A BACTERIOLOGIC STUDY OF SECONDARY INVADERS IN HOG-CHOLERA *

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Study of hog-cholera has shown that the disease is complicated by secondary invaders such as Bacillus suipestifer, B. suisepticus, B. typhi suis and B. enteritidis. The first organism has been noted so commonly in animals suffering with hog-cholera that some of the early workers attributed the disease to this bacillus. It was not until the work of De Schweinitz, Dorset, and McBryde[1] appeared that the etiology was cleared up. These investigations, followed by those of Hutyra,[2] Uhlenhuth, Haendel, and others,[3] established the feature of secondary invasion.

As yet, attempts to differentiate the organisms found in hog-cholera have not met with success. Apart from B. suisepticus, which is placed in the hemorrhagic septicemia group, none of the others can be separated sharply from B. paratyphosus B and from bacilli associated with meat poisoning. The morphologic, cultural, and serologic behavior of B. suipestifer places it in such close relation to these that a definite separation is not possible with the differential-diagnostic methods now available. The significance of this organism in hog-cholera has long been recognized. It is possible by means of B. suipestifer to produce an experimental infection giving rise to pathologic changes which apparently correspond to natural hog-cholera.

The bacillus of hog-cholera (B. suipestifer), altho present in a number of cases, is not found invariably in the organs of animals with the disease. In several outbreaks of hog-cholera studied by Smith in America, no such organisms were isolated. Preiss, in a systematic examination of 80 cases from different sources, found B. suipestifer

* Received for publication May 28, 1915.

1. Circular 41, U. S. Dept. of Agriculture, Bureau of Animal Industry, Washington, D. C., 1903.
2. Handb. d. Serumtherap., p. 42; Specielle Path. u. Therap. d. Haustiere, 1910, 1, p. 142.
3. Kolle-Wassermann, Handb. d. Pathogenen Mikroorganismen, 1913, 6, p. 325; Arb. a. d. k. Gsndhtsamte., 1914, 47, p. 1020.

TABLE 1

POSTMORTEM FINDINGS IN VIRUS HOGS

Hog	Lung	Kidney	Lymph Nodes	Intestine (ileocecal)	Organisms Isolated		
					Lung	Spleen	Intestine
1	++	+	Hemorrhagic	Inflamed	P	P.C	C
2	++	++	Hemorrhagic	Inflamed	P.C	P.C	Negative
3	+++	++	Hemorrhagic	Inflamed	P	P	C
4	+++	++	Hemorrhagic	Inflamed	C	P	Negative
5	++	+	Hemorrhagic	Inflamed	P	P	Negative
6	++	+	Hemorrhagic	Inflamed	Negative	Negative	C
7	+	++	Hemorrhagic	Inflamed	P	P	C
8	++	+	Hemorrhagic +	Inflamed	P.C	P	Negative
9	+	++	Hemorrhagic	Inflamed	P	P.C	C
10	+	++	Hemorrhagic (slight)	Inflamed	Negative	P	Negative
11	+	+	Hemorrhagic	Inflamed	C	Negative	C
12	+	+++	Hemorrhagic	Inflamed	C	C	C
13	+	+	Hemorrhagic	Inflamed	Negative	C	Negative
14	+	+	Hemorrhagic	Inflamed	P.C	C	S
15	+	+	Hemorrhagic	Inflamed (slight)	C	C	C
16	+	+	Hemorrhagic	Inflamed	P	P	P.S
17	+	++	Hemorrhagic	Inflamed	C	C	Negative
18	+	—	Hemorrhagic	Inflamed	C	P.C	C
X	+	+++	Hemorrhagic	Inflamed	Negative	Negative	Negative
19	+++	+	Hemorrhagic	Inflamed	P.C	P.C	C
20	+	+	Hemorrhagic	Inflamed	Negative	C	C
21	+	+	Hemorrhagic	Inflamed	Negative	Negative	C.S
22	+++	+	Hemorrhagic	Inflamed	Negative	S	C
23	++	—	Hemorrhagic	Inflamed	Negative	Negative	C
24	+	+	Hemorrhagic	Inflamed	Negative	Negative	S.C
25	+	+	Hemorrhagic (slight)	Inflamed	Negative	S.C	C
26	+	++	Hemorrhagic	Inflamed	C	C	C
27	+	+	Hemorrhagic (slight)	+ (Petechiae)	P	P	C
28	+	—	Hemorrhagic	+ (Petechiae)	C	P.C	P.C
29	++++	++	Hemorrhagic (slight)	+ (Petechiae)	P	P	P.C
30	++	+	Hemorrhagic	+ (Petechiae)	Negative	C	C
31	+	—	Hemorrhagic	Inflamed	C	C	Negative
32	+	+	Hemorrhagic (slight)	+ (Petechiae)	P.C	P.C	Negative
33	++++	+	Hemorrhagic	Inflamed	P	P.C	C
34	+++	+	Hemorrhagic	Inflamed (slight)	P	P	Negative
35	++	+	Hemorrhagic	Inflamed	P	P.C	S.C
36	+	+	Hemorrhagic	Inflamed	P	P.C	Negative
37	++	+++	Hemorrhagic	Inflamed	Negative	P	Negative
38	+	+	Hemorrhagic	Inflamed	Negative	P	C
39	+	+	Hemorrhagic	Inflamed	P.C	P	Negative
40	+	+++	Hemorrhagic	Inflamed	P	P	C
41	+	+	Hemorrhagic	Inflamed	S.C	Negative	C
42	+	+	Hemorrhagic	Inflamed	P	P.C	Negative
43	++++	—	Hemorrhagic	Inflamed	P	P	P
44	+	+	Hemorrhagic	Inflamed	P	P	S.C
45	+	+	Hemorrhagic	Inflamed	P	P	C
46	++	++	Hemorrhagic	Inflamed (slight)	P	C	C
47	+	+	Hemorrhagic	Inflamed	Negative	Negative	C
48	+	—	Hemorrhagic (slight)	Inflamed	Negative	Negative	C
49	++	+	Hemorrhagic	Inflamed	Negative	C	C
50	+	+	Hemorrhagic	Inflamed	C	C	C
51	++	+++	Hemorrhagic (slight)	Inflamed (slight)	P	P	C
52	+++	++	Hemorrhagic	+ (Petechiae) and Ulcers	P	P	C
53	+	+	Hemorrhagic	Inflamed	Negative	Negative	C
54	+	++++	Hemorrhagic	Inflamed	C	Negative	C
55	+++	++	Hemorrhagic	Inflamed (Ulcerations)	P	P	P

+ = petechiae present. ++ = well-developed. +++ = marked. ++++ = extreme.
P = paratyphoid group. C = colon group. S = B. suisepticus.

31 times. Uhlenhuth was able to isolate the organism in 76 of 178 cases. Extraordinary numbers of such bacilli are found in pure culture in the organs of hogs artificially infected with the filtered virus of hog-cholera. From these findings, we are led to infer that normal animals harbor this bacillus; yet, of several hundred normal hogs studied by Uhlenhuth, Hübner, and others, only 8.4 percent showed what appeared to be true B. suipestifer. These figures are based on a study of the intestinal contents of the animals.

In the investigation here reported, an attempt was made to classify the chief groups of organisms found in a number of hogs artificially infected with the hog-cholera virus. The lungs, spleen, and intestine of each animal were studied in an effort to correlate the bacterial findings, with the organic lesions. The hogs were so-called virus hogs, inoculated with virus for the production of serum. The animals were killed usually on the eighth or tenth day after inoculation, when the previously observed maximal temperature was on the decline.

TECHNIC

Immediately after bleeding, the organs of each animal were excised and placed in separate containers. With a heated scalpel a small area of the lung and spleen was scorched, the organ broken at this point, and a sterilized platinum needle inserted into the tissue. Smears were then made on Conradi-Drigalski agar plates. In isolating organisms from the intestine, care was taken to avoid fecal contamination. An area was scorched with a heated scalpel and an incision made into the mucosa. With a sterile platinum spatula, the mucous membrane was separated from the serosa and a platinum needle inserted between. Material thus obtained was streaked on Conradi-Drigalski plates. After forty-eight hours' incubation, two colonies were fished from each plate and inoculated on agar slants. Further purification was effected by streaking new plates from each of these cultures, and finally transplanting to agar slants from the colonies developed. These agar cultures were used in the inoculation of the series of differential media.

The media used were Loeffler's malachite green dextrose lactose nutrose solution (Loeffler 1); Loeffler's malachite green lactose nutrose solution (Loeffler 2); Barsiekow's dextrose lactose nutrose litmus solution (Barsiekow 1); Barsiekow's lactose nutrose litmus solution (Barsiekow 2); Hetsch's litmus nutrose mannite solution; Petruschky's litmus whey (Lacmusmolke); and milk, peptone, dextrose, lactose, orcein agar, and neutral red agar.

The inoculated series were incubated at 37.5 C. for one week; then indol tests were made and the other cultural characters noted.

If the data for two of the organs examined are tabulated according to the severity of the lesions, we note the following percentage of cases in which the different bacterial groups were found:

TABLE 2

PERCENTAGE OF CASES IN WHICH THE DIFFERENT BACTERIAL GROUPS WERE FOUND IN LUNGS AND INTESTINE

Bacterial Groups	Lesions in Lungs					Lesions in Intestine				
	Few Petechiae (28)	Well-developed Petechiae (17)	Marked (7)	Extreme (3)	Total	Slight Inflammation (4)	Moderate Inflammation (44)	Petechiae (5)	Petechiae and Ulcers (2)	Total
Negative.....	9(32.1%)	6(35.3%)	1(14.3%)	0	16	1(25.0%)	12(27.2%)	1(20.0%)	0	14
B. paratyphosus.....	9(32.1%)	10(58.8%)	5(71.4%)	3(100%)	27	0	2 (4.5%)	2(40.0%)	1(50.0%)	5
B. coli.....	12(42.8%)	4(23.5%)	2(28.5%)	0	18	3(75.0%)	29(65.5%)	4(80.0%)	1(50.0%)	37
B. suisepticus.....	.....	.....	.....	.....	.....	0	6(13.6%)	0	0	6

The so-called secondary invaders do not seem at first glance to play an important part in inducing tissue changes, if we may judge from the findings in organs which show lesions of varying degree. The lungs, for example, in sixteen cases were sterile; yet they showed petechial hemorrhages and inflammation. On the other hand, the paratyphoid group appears to modify the course of the infection. Table 2 shows how these bacteria, when present in the lungs, were

TABLE 3

LOCALIZATION OF BACTERIAL GROUPS IN DIFFERENT ORGANS

Organ	B. coli	B. paratyphosus	B. suisepticus	B. coli and B. paratyphosus	B. paratyphosus and B. suisepticus	B. coli and B. suisepticus	Negative
Lung.....	11	17	..	10	..	1	16
Spleen.....	11	20	1	12	..	1	10
Intestine.....	30	3	1	1	2	3	14

correlated with a progressive intensity of the lesions. Other factors are present, however, and cannot be eliminated. The colon bacilli are found rather frequently (see Table 3). In the intestine, these organisms may play a more important part since they are naturally associated with the tract. It is possible that they are drawn into the lungs during the bleeding as a result of forced respiration and the flow of blood. The simplest explanation of the presence of these bacteria in the organs is that of metastasis by way of the circulation. The organ-

isms can enter the blood stream readily through ruptured capillaries in the intestine, for example, and so become localized in the different organs.

The frequency with which different groups of organisms were associated with the organs is shown in Table 3. The bacillus coli and bacillus paratyphosus groups were evenly distributed in the lungs and spleen, occurring each alone and in combination. In the intestine, B. coli was found most frequently (30 times) and B. paratyphosus in but three instances. B. suisepticus was isolated in nine cases of a total of fifty-five, the intestine harboring this organism in six cases.

The presence of B. suisepticus in hog-cholera does not point, according to some investigators, to an infection with what is known as true swine plague. Uhlenhuth and his co-workers[3] state that organisms resembling and behaving like B. suisepticus are found normally in the upper respiratory passages and secondarily in the lungs. These bacilli may localize themselves in other organs.

This study does not point to B. suisepticus as a very frequent inhabitant in either normal or diseased hogs. Tables 1 and 2 show that there was apparently no causal connection between the presence of this organism and the severity of the lesions. The intestines of the animals from which B. suisepticus was isolated showed inflammation and were free from petechiae. No such strains were isolated from intestines having petechiae or ulcers or both.

In an investigation of this nature, we are confronted with the problem of correlating the bacterial findings with our knowledge of what constitutes true hog-cholera. If we accept the classification made by Schern and Stange,[4] this becomes simpler. They divide hog-cholera into different groups as follows: virus cholera or "viruspest," a disease caused by virus alone; paracholera or "parapest," caused by B. suipestifer and other organisms; and hog-cholera or "pest," caused by virus, B. suipestifer, and other organisms. Bearing this classification in mind, we can more readily correlate the presence or absence of certain bacteria from organs with extensive lesions or none at all, since it is hardly to be expected that there should be no overlapping of these groups.

CHARACTERISTICS OF ISOLATED STRAINS

In addition to the cultural tests made of these organisms, motility and reaction to Gram's stain were studied. All but ten strains were

4. Ztschr. f. Infektionskrankh. d. Haustiere, 1914, 15, p. 1.

found to be motile bacilli. Of these latter, nine were ovoid, bipolar, gram-negative, non-motile organisms, and one was a gram-negative coccus, coagulating milk. This last-named organism was isolated from the intestine of one of the animals.

SUMMARY BY GROUPS

B. coli	B. paratyphosus	B. suisepticus
132	106	9

The paratyphoid strains may be correlated according to their action on Hetsch's mannite nutrose litmus solution and their indol production in peptone water, as follows: Of 106 strains, 88 (83 percent) were indol-negative, producing slight gas; 4 (3.8 percent) were indol-negative, producing abundant gas; 9 (8.5 percent) were indol-positive, producing slight gas; and 5 (4.7 percent) were indol-positive, producing abundant gas.

Thus it is seen that the organisms of this group were chiefly slight gas formers which did not produce indol. They were found principally in the lungs and spleen. Those which formed gas abundantly were divided evenly between indol-positive and indol-negative groups. The few organisms which produced indol from peptone with slight gas formation in mannite were members of the paratyphoid B group. Indol production is not generally ascribed to paratyphoid organisms, altho positive findings have been noted in some instances. Poppe,[5] using different kinds of peptone, found that some paratyphoid varieties were not indol-positive. Ordinary peptone water showed a positive reaction after four to five days. Andrejew,[6] working with a series of paratyphoid B strains, repeatedly obtained positive indol reactions.

B. paratyphosus A, and B. paratyphosus B can be distinguished by their behavior in mannite nutrose litmus solution and Petruschky's litmus whey. The A type does not ferment mannite with production of gas, but gives rise to permanent acidity in litmus whey. The B type produces both acid and gas in the Hetsch medium, and gives to litmus whey first an acid, and later a strongly alkaline reaction, leaving the media deep blue. Within two weeks or so a change to acidity takes place again. Some paratyphoid A strains can produce a small amount of gas in mannite. The few here isolated were doubtless of this type.

5. Ztschr. f. Infektionskrankh. d. Haustiere, 1908, 5, p. 42.
6. Arb. a. d. k. Gsndhtsamte., 1910, 33, p. 1030.

PARATYPHOID STRAINS FORMING INDOL AND THEIR REACTION IN LITMUS WHEY

Lung		Spleen		Intestine	
Acid	Alkaline	Acid	Alkaline	Acid	Alkaline
3	0	3	5	2	2

The indol-forming paratyphoid strains were about evenly divided with regard to their reaction in litmus whey. The spleen was found to contain the true paratyphoid B type more frequently than either the lungs or intestine. Acid formation in litmus whey with the production of indol from peptone may be looked upon as a variation. After four weeks incubation in litmus whey, these cultures were still sharply acid. Any change in the reaction of this very sensitive indicator has been noted invariably within one week. Any possibility that these strains were B. suipestifer is eliminated because of their indol-producing character, notwithstanding the fact that the acid reaction was conformable to the behavior of this organism.

The paratyphoid strains may be grouped from the standpoint of reaction to litmus whey and gas production in Hetsch's mannite solution, as follows: Of 106 strains, 90 were whey-alkaline, producing slight gas in mannite; 10 were whey-acid, producing slight gas in mannite; 5 were whey-alkaline, producing abundant gas; and 1 was whey-acid, producing abundant gas. This grouping tends to throw the greatest number of the organisms into a class of gas formers which give an alkaline reaction in Petruschky's medium. Practically all these produced but a slight amount of gas. A few gave an acid reaction in whey and produced little or no gas in the mannite medium. One of these formed no indol and was culturally like B. paratyphosus A.

CLASSIFICATION OF ISOLATED STRAINS

COLON GROUP

(1) *Typical B. coli.*—Red colonies on Conradi-Drigalski agar; gas in dextrose and lactose; gelatin not liquefied; indol-positive, non-spore-forming, motile.

Variant A.—Blue colonies on Conradi-Drigalski agar; same cultural characteristics as type; milk not coagulated. Isolated from spleen (one strain).

PARATYPHOID GROUP

(1) *Typical B. paratyphosus.*—Blue colonies on Conradi-Drigalski agar; gas in dextrose; no gas in lactose; non-spore-forming, motile. The "A" type does not form gas in mannite, does not produce indol. The "B" type forms gas in mannite and may produce indol from peptone water.

STRAINS ISOLATED FROM VIRUS HOGS

From spleen (one strain): Acid but no gas in mannite; litmus whey, acid; indol-negative.

From intestine (one strain): Acid but no gas in mannite; litmus whey, acid; indol-positive.

From lung (45), spleen (38), intestine (2): Acid and gas in mannite; litmus whey, alkaline; indol-negative.

From spleen (5), intestine (2): Acid and gas in mannite; litmus whey, alkaline; indol-positive.

From lung (3), spleen (3), intestine (1): Acid and gas in mannite; litmus whey, acid; indol-positive.

From lung (2), spleen (2), intestine (1); Acid and gas in mannite; litmus whey, acid; indol-negative.

SUMMARY AND CONCLUSIONS

Organisms belonging to the bacillus paratyphosus group were chiefly associated with the lungs and spleen of hogs infected with the virus of hog-cholera.

Bacillus coli was frequently found in the lungs and spleen, either alone or in combination with organisms of the paratyphoid group.

Bacillus suisepticus was isolated in few cases—but 9 out of 55—and was found chiefly in the intestine.

Classification of the organisms shows that the greatest number belonged to the paratyphosus B group. The majority of these did not form indol and were found chiefly in the lungs and spleen.

Bacterial findings did not appear to be correlated with the lesions observed in different organs.

The significance of secondary invaders in hog-cholera is not apparent from a study of the lesions and the different groups of organisms isolated.